Physiology & Behavior 143 (2015) 158–165

Contents lists available at ScienceDirect

Physiology & Behavior

journal homepage: www.elsevier.com/locate/phb

Strain variation in the adaptation of C57Bl6 and BALBc mice to chronic hypobaric hypoxia

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HIGHLIGHTS

- 2 mouse strains were exposed to high altitude (HA) for 12 weeks.
- HA exposure induced weight loss recovered poorly in BALBc and C57Bl6 strains.
- Performance on rotarod and treadmill improved with HA exposure.
- Plethysmography showed increased respiratory frequencies and tidal volumes with HA.
- Whole body metabolic rates increased in both strains, particularly in BALBc mice.

ARTICLE INFO

Article history:
Received 15 September 2014
Received in revised form 23 December 2014
Accepted 30 January 2015
Available online 31 January 2015

Keywords:
Hypobaria
Hypoxia
Stress
Adaptation
Mouse strains
High altitude

ABSTRACT

The interplay of environmental and genetic factors may lead to a spectrum of physiological and behavioral outcomes. How environmental stress factors interact with the diverse mouse genomes is still poorly understood and elucidating the underlying interactions requires specific stress models that can target integrated physiological systems. Here, we employ behavioral tests and whole-body plethysmography to examine the effects of 12 weeks of simulated high altitude (HA) exposure on two inbred mouse strains, BALBc and C57Bl6. We find that HA induced-weight loss recovers at significantly different rates in these two strains. Even at 12 weeks, however, both strains fail to reach body weight levels of controls. Performance on two motor tasks, rotarod and treadmill, improve with HA exposure but more prominently in BALBc mice. Whole-body plethysmography outcomes indicate that compensation to chronic HA includes increased respiratory frequencies and tidal volumes in both strains. However, the effects on tidal volume are significantly greater in BALBc mice and showed a biphasic course. Whole- body metabolic rates are also increased in both strains with prolonged HA exposure, but were more pronounced in BALBc mice suggestive of less successful adaptation in this strain. These adaptations occur in the absence of gross pathological changes in all major organs. Together these results indicate that chronic HA exposure results in environmental stressors that impact the specific physiological responses of BALBc more than C57Bl6 mice. Thus, these strains provide a promising platform for investigating how genetic backgrounds can differentially reinforce the effects of long-lasting environmental stressors and their potential to interact with psychological stressors.

1. Introduction

Stressors may be presented through multiple modalities, such as harsh environmental conditions or psychological trauma, and lead to adverse physiological adaptations. The potential effect of environmental stress has been highlighted in recent conflicts in Iraq and Afghanistan where soldiers often operate under extreme temperature or altitude changes [25,37,38]. The environmental stressors and the resulting physiological adaptation may act to leave an individual more susceptible to developing post-traumatic stress disorder. Establishing such a link between genetics and sensitivities to external stressors, however, requires a stable platform to investigate the interactions of environmental and psychological stressors. Here, we significantly expand a mouse model of chronic environmental stress, 12 weeks of exposure to a simulated altitude of 5000 m, using two frequently investigated mouse strains, BALBc and C57Bl6. These strains have been previously implicated as important susceptibility and resiliency models able to

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http://dx.doi.org/10.1016/j.physbeh.2015.01.036

unravel genetically driven differential responses to psychological and environmental stressors [21,24,27,36]. However, these studies investigated adaptations over a relatively short time duration. Here we expand upon these results to examine a timespan where, in addition to acute effects, long-term adaptation to environmental stress can be investigated.

Hypoxia associated with high altitudes presents a significant environmental/physiological stressor that drives a host of adaptive responses such as alterations in body weights, ventilatory parameters and angiogenesis with a magnitude that varies with mouse strain [31,33,40]. Consistent with these studies, we find that C57Bl6 and BALBc mice exposed to both hypoxia and hypobaria show similar signs of physiological stress upon ascension to a simulated high altitude (HA) of 5000 m. By extending the duration of HA exposure to 12 weeks, we were able to expand upon previous findings and detect significant differences in the manner and degree to which each strain adapts to the chronic hypobaric–hypoxic environment. Most notably, changes in respiratory frequency and tidal volume as well as oxygen consumption and carbon dioxide production increased but remained stable in C57Bl6 mice. In contrast, the same parameters varied with exposure duration in BALBc mice in a manner that suggests a slower or less complete adaptation to the chronically stressful environment. Thus, our results demonstrate significant strain differences in responses to prolonged physiological stress, and validate these strain selections as a promising model for determining genetic contributions to multi-modal long-term stress adaptation.

2. Materials and methods

2.1. Animals and hypobaric chamber

Male mice at 7 weeks of age were ordered from Charles River (BALBc) or Jackson Laboratories (C57Bl6). Mice were housed on a reverse light cycle for at least 1 week prior to the start of data collection. All experiments were approved by the IACUC at Uniformed Services University of the Health Sciences (USUHS). Mice were housed in groups of 3 to 5 mice per cage upon arrival and then randomly divided into two groups: control and high altitude (HA) exposed. Both groups were kept on a reversed 12 h light cycle with lights on at 7 PM. HA exposed mice were placed within their home cage in a custom built hypobaric chamber (Reimers Systems, Inc. Lorton, VA). A vacuum pump (Welch Model 2585B) reduced the atmospheric pressure to ~7.4 psi or 5000 m at a rate of 200 m/min. Cage changes, including food and water, were performed once per week at normobaric normoxia. Internal CO_2 and O_2 levels as well as temperature and relative humidity were monitored and digitally recorded using a sensor bank located inside the chamber and connected to a dedicated computer. Barometric pressure was also digitally recorded using a digitizing manometer. Control mice were housed in a separate room on a reversed light cycle and experienced similar routines as exposed mice. Behavioral tests and physiological measurements were conducted prior to HA exposure to establish a pre-exposure baseline and then repeated at intervals of 24 h and 1, 3, 8 and 12 weeks following exposure onset.

2.2. Rotarod

Mice were placed individually in one lane of an Ugo Basile Rota-Rod (Ugo Basile, Varese — Italy), and were given a one-minute acclimation session at 5 rpm on the first exposure followed by 3 repetitions of acceleration from 5 to 60 rpm over 5 min. Mice were placed back on the rotarod after falling off during acclimation sessions. The three measured latencies to fall during acceleration were averaged to produce a final value.

2.3. Treadmill

Prior to the baseline measurement, mice were acclimated to the treadmill (Columbus Instruments, Columbus OH) for 5 min with the belts and shock grids off. The mice were then returned to their home cage for a minimum of 30 min prior to testing. The test phase consisted of a 5 min warm-up period of 7 m/min at an incline of 10° with the shock grids engaged at 0.87 mA stimulating at 2 Hz. The incline was then increased to 20° and the treadmill speed increased 1 m/min every 30 s. The session ended when the mouse spent three 2 second periods or a single 5 second period on the shock grid. The treadmill belt speed and running times were recorded for each session.

2.4. Metabolic and respiratory measurements

Mice ($n = 8$ per group) were placed in sealed Plexiglas tubes (500 ml in volume) that received a controlled rate of inflow air from an air-pump (0.5 l/min). Following a 10 minute acclimation period, air from four individual chambers were sampled serially by CO_2 and O_2 sensors (OxyMax, Columbus Instruments) to compute oxygen consumption (VO_2) and carbon dioxide production (VCO_2). Each sensor was calibrated to gas standards prior to the sample recording. A series of five samples from each chamber was analyzed over the course of ~1.5 h. Immediately following this procedure, the inflow air was discontinued and the chambers were made air-tight. At least two 1-minute samples of respiration-generated pressure waves were recorded from each mouse at the end of the gas sampling session. Calibration factors for each chamber were generated after the recording session using a constant pressure source. Pressure waves were digitally recorded using Respiration Frequency Monitors (Columbus Instruments, Columbus OH) at 1000 Hz using LabChart software (ADInstruments, Colorado Springs, CO) on a personal computer for offline analysis.

2.5. Isolation and analysis of resting breathing data

Digitized respiration-generated pressure waves were imported into Igor (Wavemetrics, Portland OR) and analyzed with custom written routines. The peak and trough of each breathing cycle was located from the first derivative of the pressure wave. The inspiratory time (T_i) was measured as the interval between a trough and its immediately following peak. The inspiratory volume (V_i, Fig. 1) was measured as the magnitude of the pressure change between these two points after multiplication by an equipment scaling and calibration factor [32]. Resting breathing behavior was isolated from higher frequency exploratory sniffing by plotting the V_i versus T_i for each breath. This analysis revealed two distinct clusters corresponding to high and low frequency breathing behavior (see representative examples in Fig. 1C–F). A box limiting the low frequency events corresponding to a cluster of longer inspiratory times was established and a custom written routine in Igor calculated the average T_i, V_i, and respiratory frequency (f) for these breaths. The average values were calculated for each mouse at each time point (baseline, 24 h, 1, 3, 8 and 12 weeks).

2.6. Blood collection

Whole blood samples were collected through cardiac punch under isoflurane anesthesia or submandibular vein puncture using 5.5 mm Goldenrod Animal Lancets (MEDIpoint, Inc, Mineola NY). Samples were placed into EDTA-coated plastic tubes (BD, Franklin Lakes, New Jersey) and shipped for analysis to BioReliance Corp. (Rockville, MD) or Charles River Research Animal Diagnostic Services (Wilmington, MA) for complete blood count (CBC) with differential analysis.

2.7. Pulse oximetry

Within 24 h prior to the onset of HA exposure, mice were briefly anesthetized with isoflurane and the fur around the neck was removed by first shaving the region with an electric razor followed by application of a depilatory (Nair, Church and Dwight Co., Inc., Ewing NJ). After thoroughly rinsing the neck to remove all traces of depilatory, an adult

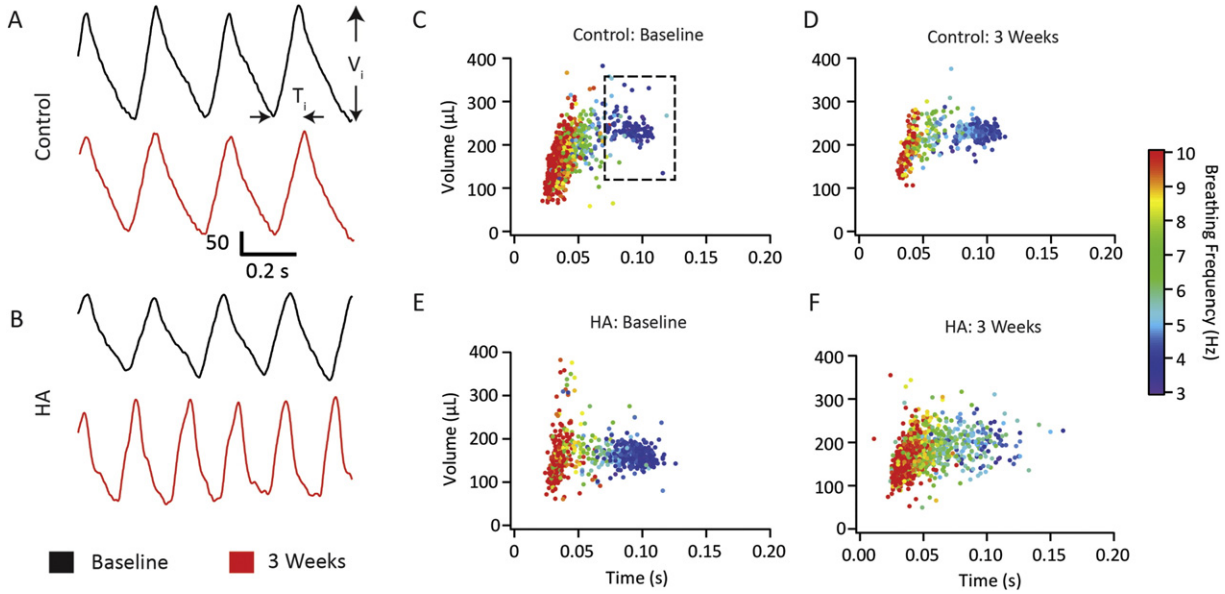

Fig. 1. Technique for isolating resting breathing epochs from whole-body plethysmography recordings. Representative raw data traces from control (A) and 5000 meter exposed (B) BALBc mouse. Black traces were baseline measurements and red traces were recorded following either three weeks at sea level (control, A) or exposure to hypobaria/hypoxia (5000 m, B). (C–F) Plots of respiratory volume versus inspiratory time for each breath revealed two distinct clusters each associated with a distinct breathing frequency. The lower frequency cluster (outlined in C, 3 to 5 Hz) correlated with resting breathing and was unchanged after three weeks at sea level (C, D). In contrast, the frequency of breaths in this cluster was significantly increased following exposure to hypobaria/hypoxia (E, F).

mouse pulse oximetry collar was placed around the neck and attached to a MouseOx pulse oximeter system (Starr Life Sciences Corporation, Oakmont, PA). Mice were then housed individually in a Starr Life Sciences conscious subjects enclosure and measures of percutaneous arterial blood saturation (SpO_2), pulse rate and general activity levels were acquired at 1 Hz using MouseOx conscious subjects software and stored on a personal computer.

2.8. Pathology

Mice were overdosed with isoflurane and perfused transcardially with 1x PBS followed by 4% paraformaldehyde. The major organs were isolated and placed in 10% formalin followed by paraffin embedding. Tissue slices at 3 µm thick were stained with H&E by either the USUHS Histopathology Core or Histoserv Inc. (Germantown, MD). Microscopic evaluation for tissue damage was performed by board certified pathologists at the Armed Forces Radiobiology Research Institute (Bethesda, MD) and Global VetPathology (Montgomery Village, MD).

2.9. Data analysis

Within-strain factor analysis was assessed using two-way repeated measures ANOVAs with condition (control or 5000 m) and exposure time as the independent variables. When significant interactions were present ($P < 0.05$) post-hoc comparisons were performed using the Holm–Sidak method. Pairwise comparisons in blood test results were analyzed with Student's t-tests using a Bonferroni adjustment for repeated measures. A complete statistical analysis was performed using SigmaPlot (Systat Software, Inc., San Jose, CA).

3. Results

3.1. Physiological and behavioral adaptation at 5000 m

Upon exposure to the simulated HA of 5000 m, both BALBc and C57Bl6 mice experienced an initial drop in body weight (Fig. 2). Although exposed mice eventually began to regain weight, it took longer for BALBc mice to surpass baseline levels compared to C57Bl6 mice (arrows in Fig. 2A and B respectively). Additionally, once the recovery began, BALBc mice continued to make significant gains in body weight through the 12-week exposure ($P < 0.001$). In contrast, the body weight in C57Bl6 plateaued at 8 weeks ($P = 0.79$ for 8 vs

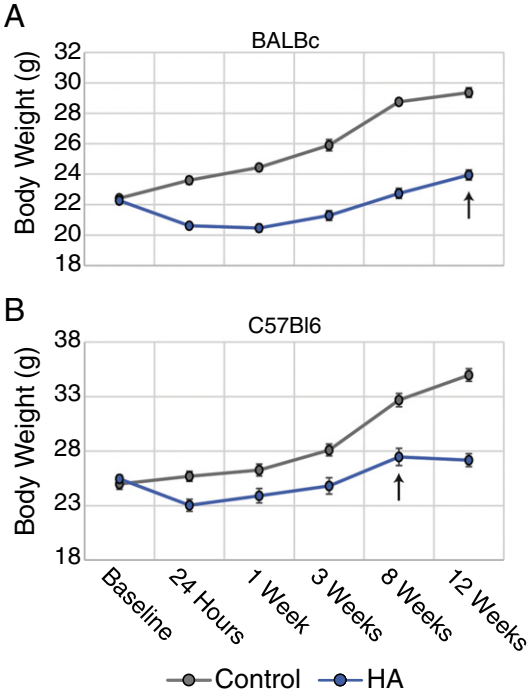

Fig. 2. High altitude exposure adversely affected normal weight gain in BALBc (A) and C57Bl6 (B) mice. Body weights of exposed mice were significantly different at all post-exposure time points ($p < 0.001$). Arrows indicate the first time point where the body weights of high altitude mice became significantly greater than their baseline values. BALBc mice took longer to achieve this milestone compared to C57Bl6 mice. Two-way Repeated Measures ANOVA with Holm–Sidak post-hoc comparisons. * = $p < 0.05$. N = 8 for all time points and conditions except for the 5000 m C57Bl6 at 12 weeks where N = 7.

12 weeks). The initial weight loss upon exposure likely resulted from a pause in eating and drinking behavior as the mice were sessile for the first 24 to 36 h of HA exposure while acclimating to the hypoxic conditions (10 to 11% O_2). The overall slower weight gains may have reflected changes in appetite or basal metabolic rates under hypoxic conditions [7,8,15,41].

To compensate for the HA exposure, hematocrit levels of exposed mice gradually increased from 53 ± 1% (n = 9) to 71.1 ± 0.9% in BALBc mice (n = 6) and 53.4 ± 0.4% (n = 6) to 78 ± 1% (n = 5) in C57Bl6 mice three weeks ($P \ll 0.001$ for both strains, Student's t-test). Within the HA exposed groups, the average hematocrit for C57Bl6 mice was significantly higher than the BALBc mice (P = 0.02). In contrast, platelet levels fell from 920 ± 85 ($\times 10^3$ cells/μL, n = 9) to 733 ± 84 ($\times 10^3$ cells/μL, n = 6) in BALBc mice (P = 0.16) and 1000 ± 110 ($\times 10^3$ cells/μL, n = 6) to 660 ± 100 ($\times 10^3$ cells/μL, n = 5) in C57Bl6 (P = 0.03) after 3 weeks. These results indicated that hematological changes, consistent with adaptation to high altitude exposure [2,16,26], were present in both strains but were significantly greater in C57Bl6 compared with BALBc mice at these time points. Functionally, we observed a significant drop in arterial oxygen saturation (SpO_2 measured with a MouseOx Pulse Oximeter, see Materials and methods) at altitude in both strains over the first 24 h of exposure. BALBc mice (N = 4) fell from a baseline of 98 ± 3% to 70 ± 4% after 1 h at altitude and further declined to 64 ± 4% after 24 h of exposure. C57Bl6 mice (N = 2) fell from a baseline SpO_2 of 96 ± 4% to 61 ± 4% and 58 ± 4% after 1 and 24 h at altitude respectively. Heart rates showed an initial decline after 1 h at 5000 m decreasing from a baseline of 640 ± 40 beats per min (bpm) to 480 ± 30 bpm in BALBc mice and, in C57Bl6 mice, from a baseline of 700 ± 60 bpm to 460 ± 60 bpm. After 24 h at altitude, both strains showed a slight recovery in heart rates increasing to 520 ± 50 bpm in BALBc mice and 470 ± 70 bpm in C57Bl6. One mouse from each strain was examined at either 2 weeks (BALBc) or 3 weeks (C57Bl6) of HA exposure. At these time points, the heart rates had continued to recover reaching 700 ± 20 bpm in the BALBc mouse and 690 ± 15 bpm in the C57Bl6 mouse. Arterial oxygen saturation levels remained low at 62 ± 4% in the BALBc mouse and 51 ± 3% in the C57Bl6 mouse.

Samples from heart, lung, kidney, liver, spleen and brain were assessed for pathological changes using H&E staining of paraffin embedded tissue. No change was observed in any tissue at any of the time points (1, 7 and 21 days of exposure) suggesting that prolonged HA exposure does not cause overt changes in tissue structure, inflammatory responses or cell death.

Consistent with a lack of pathological changes in major organs, we found that HA exposure improved the performance of mice on the rotarod and treadmill. We measured performance on these tests at baseline, 24 h and 1, 2, and 3 weeks post exposure for the rotarod and out to 12 weeks for the treadmill and compared them to unexposed controls. There was no significant interaction between time spent in the chamber and experimental group for either test; therefore, all time points after the baseline recordings were consolidated. In both mouse strains, HA exposure improved performance on the rotarod by significantly increasing the latency to fall (Fig. 3A, BALBc: 107 ± 11 s vs. 136 ± 15 s; C57Bl6: 176 ± 11 s vs. 211 ± 13 s; P = 0.005 and 0.04, respectively). The rotarod measures both physical stamina and motor coordination, but allows mice to terminate the test relatively penalty-free (i.e. by falling off the rotating rod). Thus, motivational factors could influence the overall performance. The treadmill, however, delivers a mild tail or foot shock to the mice upon leaving the belt encouraging them to remain active to a greater extent. In this test, HA-exposed BALBc mice significantly improved their performance following HA-exposure in comparison to controls (Fig. 3B, 580 ± 37 s vs. 842 ± 43 s, P = 0.0004). In contrast, the improvement in HA exposed C57Bl6 on the treadmill was not statistically significant (Fig. 3B, 567 ± 22 s vs. 651 ± 27 s, P = 0.19). These results suggested that HA-exposure positively affected physical stamina in BALBc mice, but not in C57Bl6 mice. Whether or not other mechanisms related to motivation were influenced with exercise stress needs further investigation.

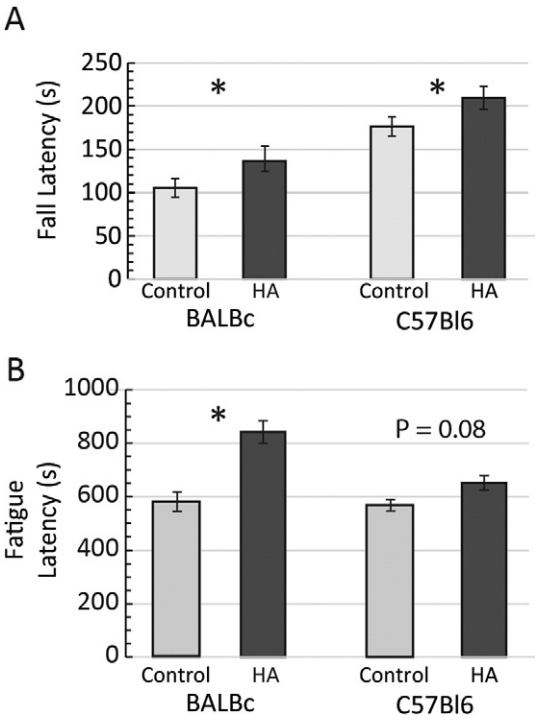

Fig. 3. Differential effects of high altitude exposure on motor performance in BALBc and C57Bl6 mice. In both strains of mice, HA exposure significantly improved performance on the rotarod by increasing the latency to falling off the rotating rod (A). On the treadmill (B), BALBc mice showed a greater improvement in performance following HA exposure in comparison to similarly exposed C57Bl6 mice. Asterisks indicate significant differences of $P < 0.05$, Student's t-test).

3.2. Comparative changes in ventilation parameters

Beyond the hematological and motor performance changes, a strain-dependent differential adaptation to chronic hypobaric hypoxia occurred. To better understand these interactions, we assessed how chronic HA exposure affected the metabolism and respiration of C57Bl6 and BALBc mice following 1 day thru 12 weeks using whole body plethysmography. Fig. 1A and B show representative recordings from a control and HA-exposed BALBc mouse, respectively. Our analysis focused on quiescent breathing behavior (see Materials and methods) and inspiratory volumes (V_i) and breathing frequencies (*f*) associated with exploratory and grooming behavior were not considered here as no significant differences were observed in these measurements in either strain (data not shown).

Analysis of group data (n = 8 for each strain and each condition) revealed a significant shift toward higher breathing frequencies (*f*) in both strains of mice following HA exposure. This pattern of breathing was initiated after 1 day of HA exposure (P = 0.059 and 0.351 for BALBc and C57Bl6 respectively at day 1 vs. baseline) and persisted throughout the 12 week exposure period (Fig. 4A and B). The *f* for BALBc mice continued to increase over the course of exposure with the value at 12 weeks becoming significantly greater than that seen after 1 day post-exposure (P = 0.022). For C57Bl6, the *f* remained elevated but was stable in exposed mice through 12 weeks of HA exposure.

The average V_i response increased in HA-exposed mice of both strains, although the effect of HA exposure led to a greater V_i in the BALBc strain compared with C57Bl6 mice (Fig. 4C and D). This change in BALBc mice progressively increased with the peak occurring at 3 weeks of HA exposure at which point V_i tended to decline (Holm–

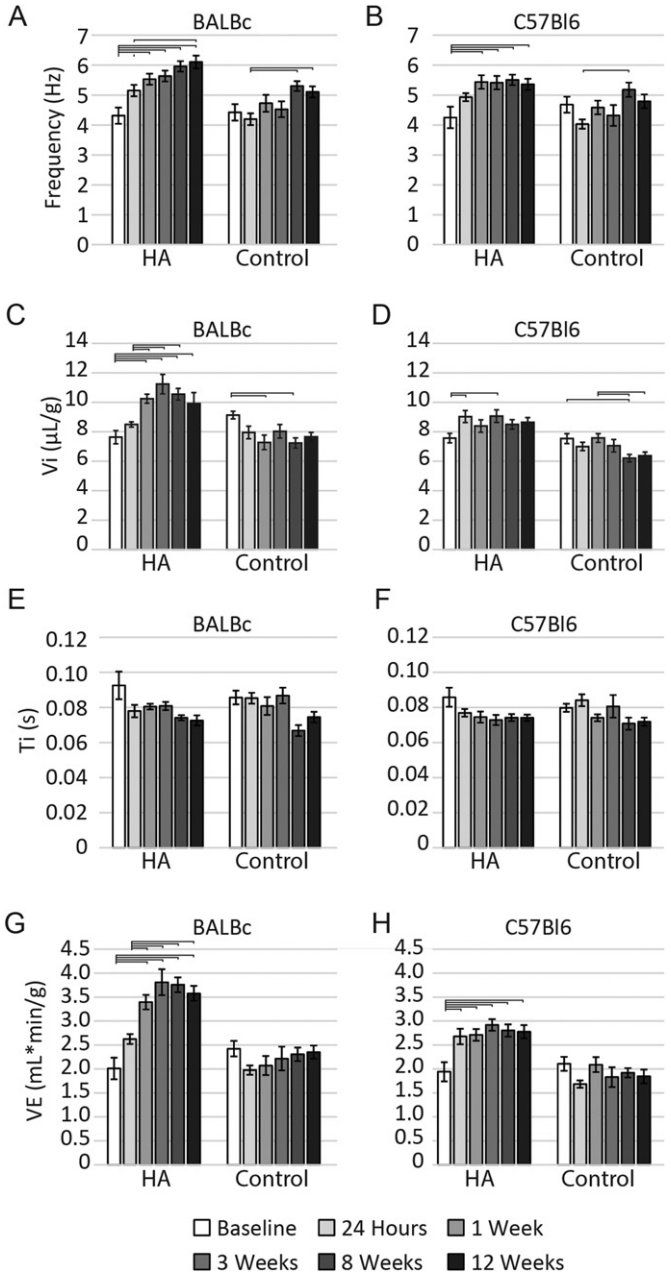

Fig. 4. Ventilation parameters are differentially altered by high altitude exposure in BALBc and C57Bl6 mice. Respiration frequency tended to increase as a function of exposure time in BALBc mice (A) while this effect plateaued early in C57Bl6 mice (B). Inspiratory volumes showed a biphasic response in BALBc mice (C), but remained comparatively minor and stable in C57Bl6 mice (D). The duration of the inspiratory phase was not significantly affected by exposure in either strain although there was a trend toward shorter inspiratory times (E, F). Changes in minute volumes were strongly strain dependent with BALBc mice (G) showing sharp increases with time and a delayed trend toward recovery while C57Bl6 mice again remained stable throughout the exposure (H). Statistically significant differences within a condition ($P < 0.05$) are indicated with brackets.

Sidak post-hoc comparison 3 weeks vs. 12 weeks, $P = 0.21$). The average T_i responses tended to decrease during exposure in both strains (Fig. 4E and F); however, there were no significantly detectable differences with time in either strain (BALBc, $P = 0.33$; C57Bl6, $P = 0.16$). The changes in *f* (Fig. 4A and B) with modest alterations in T_i (Fig. 4E and F) suggested that the phase of breathing related to expiration is also shorter in both strains.

The magnitude of breathing as measured by the minute volume (V_E) was also increased significantly in both BALBc and C57Bl6 mice (Fig. 4G and H). However, the effect HA exposure on V_E was significantly greater in BALBc compared with C57Bl6 mice at each time point after 1 week (Fig. 5; $P = 0.005$ at day 7 and $P < 0.001$ thereafter). The observation that BALBc mice showed a greater V_E than C57Bl6 mice suggested that the excessive ventilation immediately following HA exposure was magnified in BALBc relative to C57Bl6 mice. The augmented V_E response plateaued much earlier in C57Bl6 mice where no significant changes occurred after 1 day, while the plateau in BALBc mice was not reached until after 3 weeks.

3.3. Metabolic activity at sea level

In addition to breathing behavior, the average oxygen consumption (VO_2) and carbon dioxide production (VCO_2) was measured in each mouse. There was a significant interaction between condition and exposure time in BALBc mice for VO_2 over the 12-week period (Fig. 6A; $P = 0.029$, two-way repeated measures ANOVA). HA-exposed BALBc mice had significantly reduced VO_2 levels after 1 day compared to controls ($P = 0.045$, Holm–Sidak post-hoc comparison). At 12 weeks, HA-exposed BALBc showed elevated VO_2 levels above controls, but the effect was not significant ($P = 0.056$). Interestingly, BALBc controls reduced VO_2 levels over the 12-week time course ($P = 0.032$), while VO_2 remained unchanged in HA-exposed BALBc mice. No significant change due to HA exposure was observed for VO_2 in C57Bl6 mice (Fig. 6B; condition × time point interaction $P = 0.71$). In addition, BALBc mice tended to increase CO_2 production over the course of the HA exposure following an initial significant decline after 1 day compared to controls (Fig. 6C, $P < 0.05$ for 24 h vs. 3 and 12 weeks), and this parameter remained significantly elevated at 12 weeks (Fig. 6C; $P = 0.021$). No significant change was observed for VCO_2 in C57Bl6 mice (Fig. 6D, condition × time point interaction $P = 0.23$).

The early changes in VO_2 and VCO_2 in HA-exposed mice may be due to a period of fasting that occurs during the first 24 h of exposure, which alters metabolism in exposed mice in a way that is not seen in controls. The respiratory exchange ratios (RER), a reflection of metabolic activity, in HA-exposed mice were significantly reduced after 1 day in both strains (RER = 0.75 ± 0.01 vs 0.84 ± 0.01 and 0.79 ± 0.01 vs. 0.87 ± 0.01 for BALBc and C57Bl6 HA-exposed vs control respectively, $P < 0.001$) suggesting a greater reliance on fat metabolism in HA-exposed mice. At 12 weeks, only HA-exposed BALBc mice showed significantly elevated RERs above controls (12 week RER: 0.85 ± 0.01 vs 0.80 ± 0.01, $P = 0.02$). Thus, HA-exposed BALBc mice shifted toward a greater reliance on carbohydrate metabolism over the course of exposure. No significant differences were detected between treatment groups in C57Bl6 mice indicating that the differential shift in metabolic substrates with HA exposure was unique to BALBc mice.

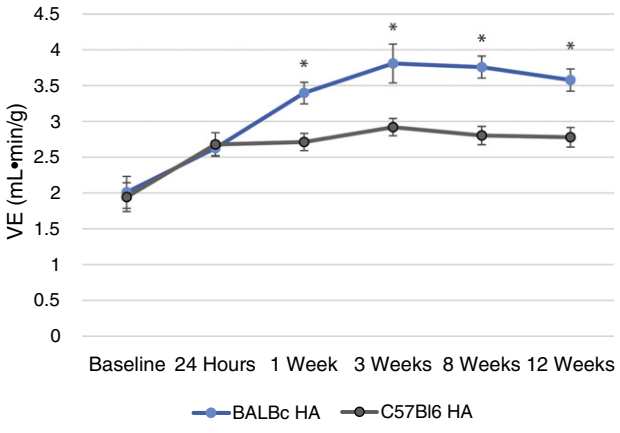

Fig. 5. Strain dependent changes in minute volumes in response to prolonged exposure to high-altitude (HA). Both strains had a similar shift in minute volumes after 24 h, but the enhancement in BALBc mice grew significantly greater than that of C56Bl6 mice as the exposure continued.

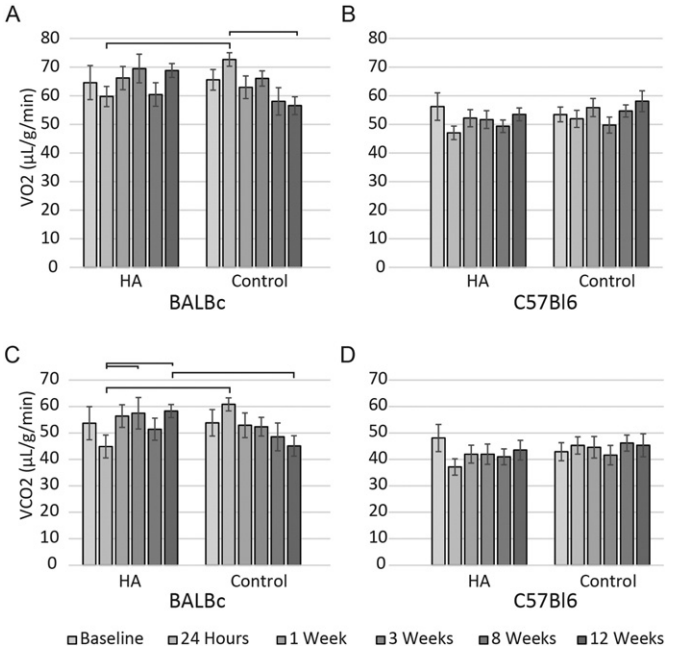

Fig. 6. Metabolic measurements remain relatively stable over three weeks of high-altitude (HA) exposure. Both VO_2 (A) and VCO_2 (B) tended to be higher in BALBc mice but, with the exception of a decline at 24 h after onset of exposure, were comparatively stable in both strains with respect to the changes seen in *f* and V_i. Brackets indicate significant differences of $P < 0.05$.

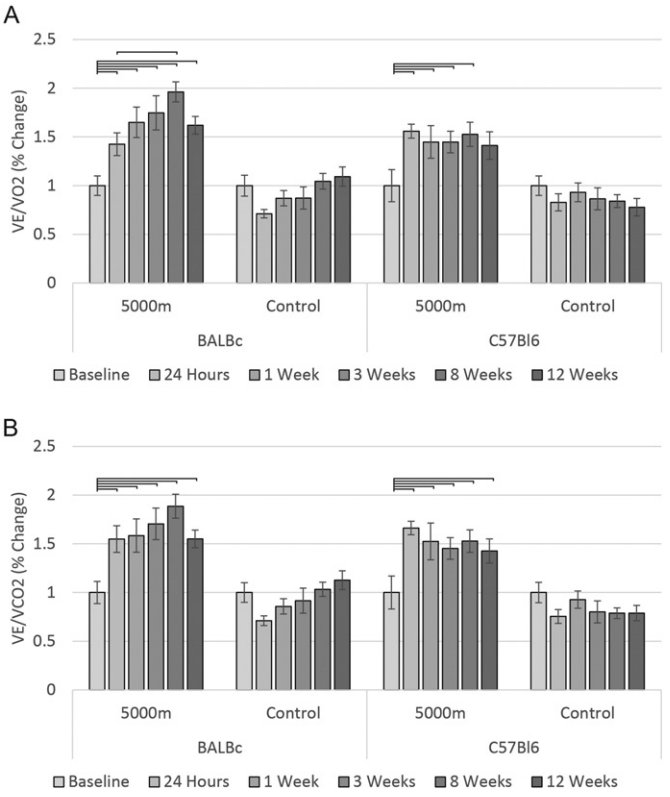

Fig. 7. Hypobaria/hypoxia induced increases in ventilatory equivalent ratios (V_{Eq}) are strain dependent. Both BALBc and C57Bl6 mice had significant increases in V_{Eq} for oxygen (A) and carbon dioxide (B) over the course of HA exposure. As seen in other respiratory parameters (*f*, V_I and V_E), the magnitude of these changes varied comparatively more with time in BALBc than in the more stable response of C57Bl6 mice.

The ratio of V_E to either VO_2 or VCO_2 is the ventilatory equivalent (V_{Eq}) and relates the ventilation to the metabolic rate (i.e., the amount of O_2 delivered and CO_2 released). Thus, the V_{Eq} for O_2 consumption and CO_2 production provide measurements of breathing efficiency. During the 12-week HA exposure, the V_{Eq} was significantly elevated in both mouse strains (Fig. 7A and B) relative to baseline V_{Eq}, suggesting that hypobaric hypoxia stimulated breathing behavior as expected. However, BALBc mice showed a progressively greater magnitude of change in both of these pulmonary measurements during the first 8 weeks, which was not seen in C57Bl6 mice. Furthermore, the BALBc mice showed an abrupt fall in V_{Eq} between 8 and 12 weeks of HA exposure, which also was not observed in C57Bl6 mice. This strain difference suggested that the stress response to chronic hypobaric hypoxia, in terms of breathing behavior, was significantly greater in BALBc compared with C57Bl6 mice. Alternatively, the adaptation to chronic HA exposure after 8 weeks was significantly delayed in BALBc mice.

4. Conclusions

Individual variation in gene expression and regulation modulate physiological and psychological acute and chronic responses to stress. Here, we expand upon previous investigations surrounding the differences between two mouse strains commonly used in hypobaric–hypoxic stress experiments, C57Bl6 and BALBc, by examining the adaptation to a simulated altitude of 5000 m for up to 12 weeks. Our results suggest that BALBc mice, which tend to demonstrate a higher sensitivity to psychological stressors paradigms [3,24,29], progressively show greater respiratory rates in response to HA conditions than similarly exposed C57Bl6 mice. Inspiratory and minute volumes in HA-exposed BALBc showed a biphasic response with time whereas the same parameters remained stable in C57Bl6 mice. The progressive increase in hyperventilatory characteristics in BALBc appears to indicate greater ventilatory distress or air hunger in comparison to C57Bl6 mice following long-term exposure to hypoxia and hypobaria. Specifically, although both strains showed increased hematocrit, HA-exposed BALBc mice showed incrementally greater *f*, V_i and V_E responses as well as V_{Eq} for oxygen and carbon dioxide compared with C57Bl6 mice. The degree to which these values varied between strains suggest that BALBc mice are more sensitive to hypobaric hypoxic stress or require a longer time period to adapt to chronic HA exposure compared with C57Bl6 mice.

Similar to what has been observed in humans [2,16,26] and multiple animal models [9,12,13,20,40], we observed a significant increase in the hematocrit of mice exposed to high altitudes for 3 weeks. We selected this length of exposure to give sufficient time for the mice to show acclimatization to chronic hypoxic exposure [22]. The HA-driven elevation in hematocrit in C57Bl6 mice was significantly higher than that observed in BALBc mice (78% versus 71% at 5000 m) suggesting that the magnitude of the hematological adaptation to HA exposure is strain-dependent and less effective in BALBc mice. A lower hematocrit in BALBc mice suggested a relatively lower capacity for carrying oxygen in the blood, and may be indicative of a more global hypoxic state at high altitude relative to C57Bl6 mice. Interestingly, BALBc mice tended to have higher SpO_2 levels at altitude compared to C57Bl6 mice possibly in compensation for a lower hematocrit.

Other measurements, such as blood gas levels, need to be measured to investigate the systemic hypoxic level more completely. Similar strain-dependent effects on hematocrit levels have been reported for rats [34] and other mouse strains not investigated here [31]. Importantly, these genetically-based differences in the hematological response to hypobaric hypoxia in animal models reflect variations in hemoglobin density found in different human populations living at high altitudes. These differences, as well as changes in resting ventilation volumes, arterial oxygen concentration and muscle capillary densities, have also been attributed to genetic variations [5,6,14,42].

Although a higher hematocrit in C57Bl6 mice suggests an increase in the O_2 carrying capacity of the blood, this also likely presents a significantly greater burden on the heart of this strain of mice due to a

greater blood viscosity. Baumann and colleagues [4] have shown very little strain variation in echocardiographic characteristics between male C57Bl6 and BALBc mice under normal conditions. Another study comparing C57Bl6 and BALBc mice suggested that these strains differ in response to myocardial infarction (MI). Of particular importance for the current study, C57Bl6 mice show a greater preservation of cardiac function following MI compared with BALBc mice [39]. Thus, whether or not the increased levels of hematocrit seen in the current study is truly beneficial remains to be determined.

In addition to increasing the oxygen carrying capacity of the blood through hematocrit changes, C57Bl6 and BALBc mice showed alterations in breathing patterns following HA exposure. When measured at sea level after different periods of HA exposure, BALBc and C57Bl6 mice showed an increase in *f* during quiescent breathing of 30% and 20%, respectively. Similar shifts in V_i were observed, but the maximum percent change occurred earlier in HA exposure at 3 weeks (32% increase in BALBc mice vs. 16% in C57Bl6). Correspondingly, V_E responses followed a similar trend showing increases of 47% in BALBc versus 33% in C57Bl6 mice compared to baseline levels. Our prolonged exposure to hypobaric hypoxia for 12 weeks makes this study unique in comparison to studies of relatively shorter exposure duration that also demonstrated strain differences in pulmonary and physiological characteristics of inbred mice. Specifically, these results support the hypothesis that BALBc mice demonstrated a significant delay in the adjustment of breathing behaviors as an adaptive response to simulated HA, which occurred between 8 and 12 weeks of exposure (Fig. 7). This rate of adjustment was more rapid for C57Bl6 mice in which the adaptive breathing response to HA exposure occurred within the first week. In both cases, the breathing behavior became more efficient over the course of exposure with respect to the metabolic rate but each strain demonstrates different recovery rates. That is, the hyperventilatory characteristic in BALBc appeared to be attenuated from an earlier indication of ventilatory distress or greater air hunger after 12 weeks. While the environmental stress of HA exposure was acting effectively in both strains, the excessive ventilatory behavior of BALBc mice seen through the first 8 weeks was alleviated with more prolonged HA exposure at 12 weeks. These results support the hypothesis that the genetic backgrounds of inbred mice C57Bl6 and BALBc would lead to differential response to this environmental stressor [31,32,40].

During HA exposure, VO_2 was not significantly affected in either mice while VCO_2 showed only minor changes and these were only significant in BALBc mice. The biggest change was observed at the 24-hour time point and likely reflects the period of metabolic depression induced by fasting that occurred at the onset of exposure. The observation that these parameters tended to decline over time in BALBc mice but were stable in C57Bl6 may reflect differences in metabolism between these two strains. Although the absolute values of VO_2 and VCO_2 were relatively unaffected by HA exposure, the V_{Eq} results for both O_2 and CO_2 were significantly altered. Interestingly, V_{Eq} showed a trend toward increasing magnitude in BALBc mice, whereas these values shifted toward recovery to baseline levels in C57Bl6 mice. The shift in V_{Eq} for CO_2 toward higher values is consistent with increased ventilation of inadequately perfused alveoli and, in humans, is correlated with increased mortality in patients with heart failure [19]. Similarly, increases in both equivalent ratios are correlated with increased mortality in patients with pulmonary hypertension [28]. Thus, prolonged exposure to HA evoked more severe signs suggestive of cardiopulmonary dysfunction in BALBc than C57Bl6 mice, suggesting the exposure is physiologically more stressful for BALBc mice.

Besides the physiological changes discussed, HA exposure altered the performance of both strains of mice on the rotarod and treadmill. The rotarod is a test of motor coordination while the treadmill focuses on physical stamina. Interestingly, both strains of mice had improved performance on both tests after only 24 h of HA exposure and this degree of enhancement did not change significantly as the HA exposure progressed. On the treadmill, BALBc mice tended to show a greater improvement following HA exposure. This result may be due to differences in adaptation to the HA environment as discussed above or in a strain dependent perception of pain between the two strains. Although the shock intensity was adjusted to be aversive but not painful it is possible that differences in the perceived degree of aversiveness may have motivated the two strains differently [17,18]. With respect to the time course of improvement, the rapid initial increase may result from the period of inactivity imposed upon the HA mice by the hypoxic conditions leaving them more rested for the 24 hour test session. The sustained improvements may result from physiological changes, such as increased hematocrit, which could confer a performance advantage. Alternatively, the hypoxic conditions may interfere with memory formation such that HA-exposed mice do not recall that the tests can be ended by non-performance and thus continue to exert greater effort. Support for this possibility comes from observations of memory deficits in humans [1,10,11,30] and animal models [23,35]. However, more investigation is required to determine the contribution of this effect to our behavioral findings conclusively.

By examining the effects of hypobaric hypoxia for three months on two commonly used mouse strains, we have extended the research into the role of genetic variability in the physiological response to long-term exposure to environmental stress. We find that BALBc mice have a more complex pattern and slower rates of adaptation to prolonged hypobaric hypoxia than C57Bl6 mice. These differences became particularly apparent following the three-week time point where BALBc mice continued to compensate while the C57Bl6 response was largely stable. Thus, as reported for psychological stress [24,27], BALBc mice appear to adapt differently than C57Bl6 mice to the environmental stresses of high altitudes. Future studies investigating which genetic variations between these two strains contribute to the differential adaptive response to environmental stressors will be important for identifying specific loci or signaling pathways, which leave individuals susceptible or resilient to pathological responses to stress.

Acknowledgments

We thank Dr. Charles A. Vacchiano, Duke University and Dr. Richard T. Mahon, Undersea Medicine Department, Naval Medical Research Center for their inspirational help at the beginning of this project. We also thank Mr. Stephen Reimers, Reimers Systems, Inc. for all his outstanding work related to the design and construction of our hypobaric chamber.

Support:

This research was supported by a grant from the Air Force Medical Support Agency and approved for public released by SAF/PA, 4 March 2015.

Disclaimer:

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